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➢ TITLE OF THE INVENTION *(280 characters max)*

A JASMONATE COMPOUND, PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE THEREOF

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Respectfully submitted,

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A JASMONATE COMPOUND, PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of jasmonate compounds. More specifically, the invention relates to jasmonate compounds for pharmaceutical compositions, and especially as chemotherapeutic agents for treatment of mammalian cancers.

BACKGROUND OF THE INVENTION

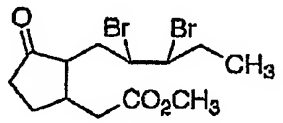
Jasmonates are a family of plant stress hormones, derived from linolenic acid by the octadecanoid pathway, and are found in minute quantities in many edible plants. Stress hormones such as the jasmonate family, have evolved in plants, and are released in such times of stress such as extreme UV radiation, osmotic shock, heat shock and pathogen attack, to initiate various cascades which end in appropriate responses. Examples of members of the jasmonate family are jasmonic acid, which is crucial to intracellular signaling in response to injury, and methyl jasmonate, which causes induction of a proteinase inhibitor that accumulates at low concentrations in response to wounding or pathogenic attacks. Jasmonates have been patented for a variety of uses in plant growth and crop improvement, but have not been previously known for use in medicine. The inventors have recently disclosed use of jasmonates for the treatment of mammalian cancer, in U.S. Patent No. 6,469,061, reference to which is incorporated hereby in its entirety. In U.S. Patent No. 6,469,061, the inventors showed that jasmonates were directly cytotoxic for various types of human cancer cells derived from breast, prostate, skin and blood cancers. While jasmonates elicited death in human leukemic Molt-4 cells, they did not damage normal lymphocytes. Subsequent data collected by the inventors similarly showed jasmonates do not damage healthy erythrocytes (see WO 02/080890). In U.S. Patent No. 6,469,061, one jasmonate compound, methyl jasmonate, was shown to be effective in preventing development of lymphomas in mice. See also Fingrut, O. and E. Flescher. 2002. "Plant stress hormones suppress the proliferation and induce apoptosis in human cancer cells", Leukemia 16: 608-616. Jasmonates have been

widely reviewed in U.S. Patent No. 6,469,061, including prior art patented uses, and a suspected mode of action.

SUMMARY OF THE INVENTION

The inventors have now synthesized a novel jasmonate compound, termed "methyl jasmonate di-bromide", or "MJDB", which is significantly more potent than the most effective jasmonate disclosed in U.S. Patent No. 6,469,061, namely, methyl jasmonate. MJDB, as shown below, exerts selective cytotoxicity on cancerous lymphocytes drawn from patients, while sparing normal lymphocytes.

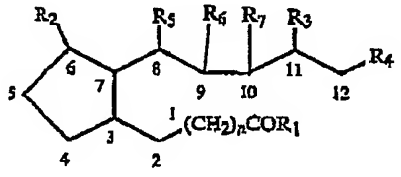
The present invention discloses a novel jasmonate compound, termed "methyl jasmonate di-bromide", or "MJDB", having Formula I:



Formula I

including salts, hydrates solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof. This compound can be utilized for any purpose known to man, and its utilization is not limited to the field of medicine.

The present invention further discloses pharmaceutical jasmonate compositions comprising a pharmaceutically acceptable carrier, and as an active ingredient a compound of Formula II:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7;

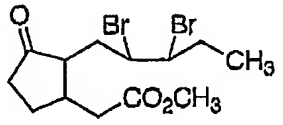
including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

In certain embodiments of the pharmaceutical composition, in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

In other embodiments, in the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

Preferably, in the pharmaceutical composition, in the compound of formula II, at least one of R_6 and R_7 is Br.

Most preferably, the compound of formula II is methyl jasmonate di-bromide, shown in formula I:



Formula I

According to some embodiments of the pharmaceutical composition, in the compound of formula II, n=0.

In other embodiments, in the compound of formula II, R_1 is a lower alkoxy.

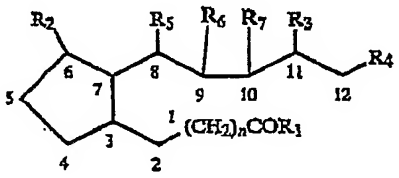
Further, in some embodiments, in the compound of formula II, R_3 and R_5 are H.

Still further, according to other embodiments, in the compound of formula II, both R_6 and R_7 are halogens.

Moreover, in additional embodiments, in the compound of formula II, n = 0, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

Preferably, in the pharmaceutical composition the active ingredient is dissolved in any acceptable lipid carrier.

The present invention additionally discloses a method for reduction of the growth of mammalian cancer cells, comprising applying to said cancer cells a therapeutically effective amount of a compound of the Formula II:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7;

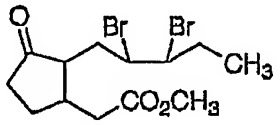
including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

The term "reduction of growth" in relation to cancer cells, in the context of the present invention refers to a decrease in at least one of the following: number of cells (due to cell death which may be necrotic, apoptotic or any other type of cell death or combinations thereof) as compared to control; decrease in growth rates of cells, i.e. the to total number of cells may increase but at a lower level or at a lower rate than the increase in control; decrease in the invasiveness of cells (as determined for example by soft agar assay) as compared to control even if their total number has not changed; progression from a more differentiated cell type to a less differentiated cell type; a deceleration in the neoplastic progress; or alternatively the slowing of the progression of the cancer cells from one stage to the next.

Reduction of growth of cancer cells may be utilized for the treatment of cancer by the administration, to an individual in need of such treatment, of a therapeutically effective amount of the compound of the present invention, as described in the claims.

In a preferred embodiment of the method for reduction of the growth of mammalian cancer cells, in the compound of formula II, at least one of R_6 and R_7 is Br.

Most preferably, in the method for reduction of the growth of mammalian cancer cells, the compound is Formula I:



Formula I

In some embodiments of the method, in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

According to other embodiments of the method, in the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

Further, in other embodiments of the method, in the compound of formula II, n=0.

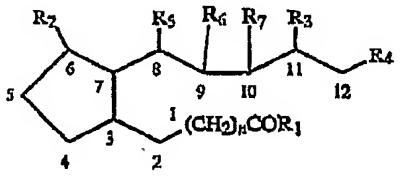
Still further, in other embodiments of the method, in the compound of formula II, R_1 is a lower alkoxy.

Moreover, in other embodiments of the method, in the compound of formula II, R_3 and R_5 are H.

Additionally, in other embodiments of the method, in the compound of formula II, both R_6 and R_7 are halogens.

Further, in other embodiments of the method, in the compound of formula II, n = 0, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

The present invention additionally discloses use of a composition of Formula II for preparing a medicament for the treatment of cancer in mammals:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

The term "treatment of cancer" in the context of the present invention includes at

least one of the following: a decrease in the rate of growth of the cancer (i.e. the cancer still grows but at a slower rate); cessation of growth of the cancerous growth, i.e., stasis of the tumor growth, and, in preferred cases, the tumor diminishes or is reduced in size. The term also includes reduction in the number of metastasis, reduction in the number of new metastasis formed, slowing of the progression of cancer from one stage to the other and a decrease in the angiogenesis induced by the cancer. In most preferred cases, the tumor is totally eliminated. Additionally included in this term is lengthening of the survival period of the subject undergoing treatment. This term also encompasses prevention for prophylactic situations or for those individuals who are susceptible to contracting a tumor. The administration of the compounds of the present invention will reduce the likelihood of the individual contracting the disease. In preferred situations, the individual to whom the compound is administered does not contract the disease.

The term "cancer" in the context of the present invention includes all types of neoplasm whether in the form of solid or non-solid tumors, from all origins, and includes both malignant and benign conditions as well as their metastasis. In particular this term refers to: carcinoma, sarcoma, adenoma, hepatocellular carcinoma, hepatocellular carcinoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphagiosarcoma, synovioama, Ewing's tumor, leimyosarcoma, rhabdotheliosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hematoma, bile duct carcinoma, melanoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependynoma, pinealoma, retinoblastoma,, multiple myeloma, rectal carcinoma, cancer of the thyroid, head and neck cancer, brain cancer, cancer of the peripherial nervous system, cancer of the central nervous system, neuroblastoma, cancer of the edometrium, myeloid lymphoma, leukemia, lymphoma, lymphoproliferative diseases, acute myelocytic leukemia, chronic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma as well as metastasis of all the above.

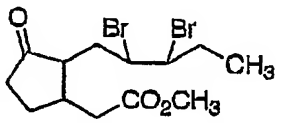
More preferably the cancer is selected from the group consisting of prostate cancer, breast cancer, skin cancer, colon cancer, lung cancer, pancreatic cancer, lymphoma, leukemia, head and neck cancer, kidney cancer, ovarian cancer, bone cancer, liver cancer or thyroid cancer. Most preferably, the cancer is selected from lymphoblastic leukemia, lung carcinoma, melanoma and colon cancer.

According to certain embodiments, during use of the composition of Formula II for preparing a medicament for the treatment of cancer in mammals, in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

According to other embodiments, the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

Further, in other embodiments, in the compound of formula II, at least one of R_6 and R_7 is Br.

Most preferably, during use of the composition of Formula II for preparing a medicament for the treatment of cancer in mammals, the compound of formula II is methyl jasmonate di-bromide:



According to other embodiments, in the compound of formula II, n=0.

Additionally, in other embodiments, in the compound of formula II, R_1 is a lower alkoxy.

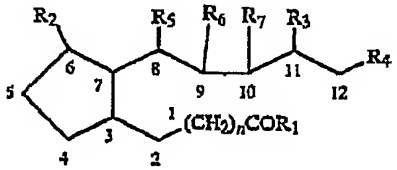
Further, in other embodiments of this use, in the compound of formula II, R_3 and R_5 are H.

Still further, in other embodiments of this use, in the compound of formula II, both R_6 and R_7 are halogens.

Additionally, in other embodiments of this use, in the compound of formula II, n = 0, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

Moreover, in other embodiments of this use, the medicament additionally comprises at least one active chemotherapeutic agent other than the compound of Formula II. In certain embodiments, the novel compound may be administered alongside with traditional chemotherapeutic drugs that are effective but have considerable side effects. The combination of the jasmonate compound and the traditional drug, may allow administration of a lesser quantity of the traditional drug, and thus the side effects experienced by the subject may be significantly lower, while a sufficient chemotherapeutic effect is nevertheless achieved.

The present invention additionally discloses a method for the treatment of cancer in mammals, comprising administering to a mammal a therapeutically effective amount of a pharmaceutical composition comprising as the active ingredient a compound of the Formula II:



Formula II

wherein:

n is 0, 1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

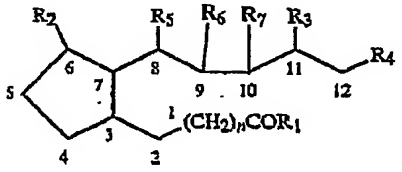
According to a preferred embodiment, the cancer is selected from the group consisting of carcinoma, sarcoma, adenoma, hepatocellular carcinoma, hepatocellular carcinoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphagiosarcoma, synovioama, Ewing's tumor, leimyosarcoma, rhabdotheliosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hematoma, bile duct carcinoma, melanoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependynoma, pinealoma, retinoblastoma,, multiple myeloma, rectal carcinoma, cancer of the thyroid, head and neck cancer, brain cancer, cancer of the peripherial nervous system, cancer of the central nervous system, neuroblastoma, cancer of the edometrium, myeloid lymphoma, leukemia, lymphoma, lymphoproliferative diseases,, acute myelocytic leukemia, chronic leukemia, Hodgkin's lymphoma, non-Hodgkins lymphoma, malignant or benign tumors of this group, and their metastasis.

More preferably, the cancer is selected from the group consisting of prostate cancer, breast cancer, skin cancer, colon cancer, lung cancer, pancreatic cancer, lymphoma, leukemia, head and neck cancer, kidney cancer, ovarian cancer, bone cancer, liver cancer or thyroid cancer.

Most preferably, the cancer is selected from the group consisting of lymphoblastic leukemia, lung carcinoma, melanoma and colon cancer.

Additionally, in a preferred embodiment of the method, the compound is administered at a dosage selected from 1μg-1000 mg/kg body weight.

There is also provided in the present invention a pharmaceutical composition for the treatment of cancer in mammals, comprising as the active ingredient a therapeutically effective amount of the compound of Formula II:



Formula II

wherein:

n is 0, 1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

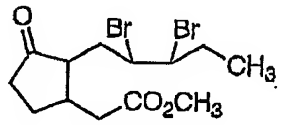
and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

The present invention further discloses a method for preparation of the compound of formula I:



Formula I

comprising:

i.adding bromine to a solution of methyl jasmonate in CCL_4 under conditions appropriate for forming the compound of Formula I;

ii.evaporating the CCL_4;

iii.obtaining the compound of formula I.

The term "pharmaceutically acceptable carrier " in the context of the present invention refers to any one of inert, non-toxic materials, which do not react with the jasmonate compound, and which can be added to formulations as diluents or carriers or to give form or consistency to the formulation. The formulation may be in the form of a pill, capsule, in the form of a syrup, an aromatic powder, and other various forms. The carrier will be selected at times based on the desired form of the formulation. The carrier may also at times have the effect of the improving the delivery or penetration of the active ingredient to the target tissue, for improving the stability of the drug, for slowing clearance rates, for imparting slow release properties, for reducing undesired side effects etc. The carrier may also be a substance that stabilizes the formulation (e.g. a

preservative), for providing the formulation with an edible flavor, etc.

The carriers may be any of those conventionally used and are limited only by chemical-physical considerations, such as solubility and lack of reactivity with the jasmonate compound, and by the route of administration. The choice of carrier will be determined by the particular method used to administer the pharmaceutical composition. Accordingly, the carrier may include additives, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. In addition, the carrier may be an adjuvant, which, by definition are substances affecting the action of the active ingredient in a predictable way.

Accordingly, pharmaceutical compositions suitable for oral administration may consist of (a) liquid solutions, where an effective amount of the active substance is dissolved in diluents, such as water, saline, natural juices, alcohols, syrups, etc.; (b) capsules (e.g. the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers), tablets, lozenges (wherein the active substance is flavored, such as with sucrose and acacia or tragacanth, or the active substance is in an inert base, such as gelatin and glycerin), and troches, each containing a predetermined amount of the active ingredient as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; (e) suitable emulsions; (f) liposome formulation; and others.

At times, the active compound may be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

Furthermore, at times, the pharmaceutical compositions may be formulated for parenteral administration and may include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Oils such as petroleum, animal, vegetable, or synthetic oils and soaps such as fatty alkali metal, ammonium, and

triethanolamine salts, and suitable detergents may also be used for parenteral administration. The above formulations may also be used for direct intra-tumoral injection. Further, in order to minimize or eliminate irritation at the site of injection, the compositions may contain one or more nonionic surfactants. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described and known in the art.

The formulations of the present invention may be administered to an individual in need at a dosage that is suited to the severity of the disease, to the age and physical condition of the individual and to the means of administration. This dosage can be determined by one familiar with the field of medicine. A preferred dosage will be within the range of 1-1000 mg/kg of body weight.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

Figure 1 is a graph illustrating MJDB is highly cytotoxic towards human leukemia cells, while it has almost no toxicity towards lymphocytes from healthy donors.

Figure 2 is a comparison of the level of toxicity effected by MJDB and by the previously studied methyl jasmonate on four human malignant cell lines originating in lymphoblastic leukemia, lung carcinoma, melanoma, and colon carcinoma.

DETAILED DESCRIPTION OF THE INVENTION

It is appreciated that the detailed description that follows is intended only to illustrate

certain preferred embodiments of the present invention. It is in no way intended to limit the scope of the invention, as set out in the claims.

EXPERIMENTAL PROCEDURES

SYNTHESIS OF MJDB

A solution of methyl jasmonate in CCl_4 at –20°C was treated with bromine until a yellow color was kept for 5 minutes. The solvent was then evaporated and the yellowish residue chromatographed on an MeOH washed Silica gel column (VLC) and eluted with hexane/5-10% EtOAc.

A 1:1 mixture of two possible racemates was obtained.

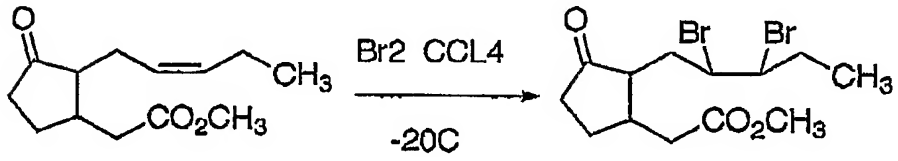
Mass spectra : m/z 384 (Br_2), Rf = 0.8 on silica gel eluted with hexane/EtOAc 1:1 .

C NMR (CDCl3) : (C-1 to C- 13) : 172.1/172.3 ; 37.1/37.3 ; 38.0/38.3 ;29.5/29.6; 38.8/39.1; 218.4/219.1; 51.7 (for both); 27.0/27.2; 57.8/55.8; 60.7/60.2; 35.7/36.1; 12.4 (for both); 51.1/51.3 ppm.

H NMR (CDCl3): 2.39-2.41(H-2 and 3) ; 1.94-2.14(H-4, H-7 and H-11) ; 2.74-2.75 (H-5); 1.61(H-8) ; 4.62 and 4.89 (H-9) ; 4.14 (H-10) ; 1.12 (H-12) ; 3.75 (OMe) ppm.

SYNTHESIS OF MJDB

1)



2)Chromatography

methyl jasmonate methyl jasmonate di-bromide

CYTOTOXICITY ASSAY USED IN EXAMPLES

Inhibition of cell proliferation was determined by the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Upon completion of a given experiment, MTS (a tetrazolium compound) at 333 µg/ml + phenazine

methosulfate (at 25 μM) was added to each well of the 96-well plate for 1 hour at 37°C. This allowed for development of a color reaction in which dehydrogenases reduce the MTS in metabolically active cells. Since the cells were not washed before the addition of MTS, there were no potentially loosely adherent or non-adherent cells that could have been problematic. Soluble MTS formazan product was measured at a wavelength of 490nm using a CERES 900 HDI ELISA reader (Bio-Tek Instruments, Inc, Highland Park, VT). Optical density is directly proportional to the number of living cells in culture. Cytotoxicity (%) was calculated in the following way: [(OD of control cells – OD of drug-treated cells)/OD of control cells]×100.

EXAMPLE 1: MJDB is highly cytotoxic towards leukemia cells, and non-toxic towards healthy lymphocytes

In order to test the toxicity of MJDB towards human leukemia cells, peripheral blood lymphocytes from chronic lymphocytic leukemia (CLL) patients were harvested. These cells were shown to contain practically 100% cancer cells, as determined by flow cytometric analysis of the CD5 and CD19 markers upon the cell surface. Peripheral blood lymphocytes from healthy donors were similarly harvested. Cells were seeded at $1.5x10^4$/well, in 96-well plates and MJDB was added for 1 day at several concentrations indicated in Figure 1. The optical density that represented viable cells was determined by the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI); an assay in which viable cells produce a colored product (for details on the protocol of this assay, see below). This assay is quantitative, as the amount of color produced is read using an ELISA reader. Cytotoxicity was calculated as the percentage of control untreated cultures, mean±SD. n = 3.

Results

Referring to Figure 1, the cytotoxicity of MJDB towards peripheral blood lymphocytes from CLL patients (represented by triangles) was plotted versus its cytotoxicity towards peripheral blood lymphocytes from healthy donors (represented by diamonds). MJDB was clearly and significantly ($P<0.05$) more cytotoxic towards peripheral blood lymphocytes from chronic lymphocytic leukemia (CLL) patients than

towards peripheral blood lymphocytes from healthy donors. MJDB is highly and selectively cytotoxic towards cancer cells from CLL patients, while cytotoxicity is minimal towards lymphocytes from healthy donors.

EXAMPLE 2: MJDB is far more cytotoxic than previously studied jasmonates, as shown on four diverse human malignant cell lines

The cytotoxicity of the novel compound MJDB (methyl jasmonate di-bromide) was compared to that of the previously studied jasmonate, methyl jasmonate (MJ), which was the most effective jasmonate disclosed in U.S. Patent No. 6,469,061. The cytotoxicity of these compounds was compared as seen when each was applied to four human malignant cell lines originating in lymphoblastic leukemia, lung carcinoma, melanoma, or colon carcinoma.

Molt-4 lymphoblastic leukemia cells (at $1.5x10^4$/well), 3LL lung carcinoma cells (at $4x10^3$/well), B16 melanoma cells (at $4x10^3$/well), or HCT116 colon carcinoma cells (at $4x10^3$/well) were seeded in 96-well plates and methyl jasmonate (MJ) or MJDB at 0.5 mM were added for 1 day. Optical density representing viable cells was determined by the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (described below). Cytotoxicity was calculated as the percentage of control untreated cultures, mean±SD. n = 3.

Results

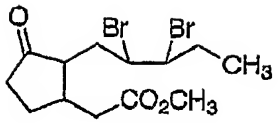
Referring to Figure 2, the percentage of cytotoxicity of methyl jasmonate (MJ, white columns) or MJDB (filled columns) is illustrated. Figure 2 clearly indicates that the newly synthesized compound MJDB is highly superior in its cytotoxic effect upon the various cancer cell lines, as compared to the previously studied methyl jasmonate. (Statistical studies showed $P<0.05$ for these results).

Thus, in summary, the novel jasmonate compound hereby disclosed, and compounds similar in nature (within the scope of the claims) can be utilized as chemotherapeutic drugs with higher potency, and a higher degree of specificity towards malignant cells, than prior art chemotherapeutic drugs. Perhaps these novel compounds will be

accompanied with fewer side-effects than previously studied chemotherapeutic drugs. Additionally, the novel compound may be administered alongside with traditional chemotherapeutic drugs that are effective but have considerable side effects. The combination of the jasmonate compound and the traditional drug, may allow administration of a lesser quantity of the traditional drug, and thus the side effects experienced by the subject may be significantly lower, while a sufficient chemotherapeutic effect is nevertheless achieved.

CLAIMS

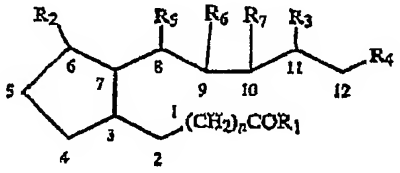
1. A compound of Formula I:



Formula I

including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

2. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, and as an active ingredient a compound of Formula II:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

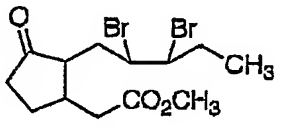
or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

3. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

4. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

5. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, at least one of R_6 and R_7 is Br.

6. The pharmaceutical composition according to claim 2, wherein the compound of formula II is methyl jasmonate di-bromide:



7. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, n=0.

8. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, R_1 is a lower alkoxy.

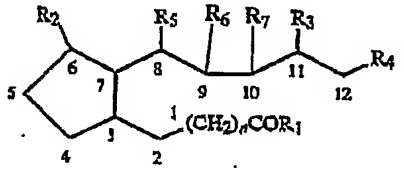
9. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, R_3 and R_5 are H.

10. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, both R_6 and R_7 are halogens.

11. The pharmaceutical composition according to claim 2, wherein in the compound of formula II, n = 0, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

12. The pharmaceutical composition according to claim 2, wherein the active ingredient is dissolved in any acceptable lipid carrier.

13. A method for reduction of the growth of mammalian cancer cells, comprising applying to said cancer cells a therapeutically effective amount of a compound of the Formula II:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

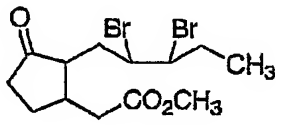
and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

14. The method according to claim 13, wherein in the compound of formula II, at least one of R_6 and R_7 is Br.

15. The method according to claim 13, wherein the compound is Formula I:



Formula I

16. The method according to claim 13, wherein in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

17. The method according to claim 13, wherein in the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

18. The method according to claim 13, wherein in the compound of formula II, n=0.

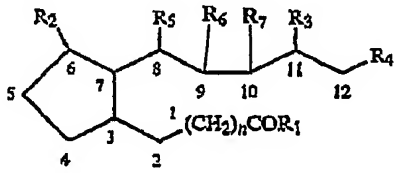
19. The method according to claim 13, wherein in the compound of formula II, R_1 is a lower alkoxy.

20. The method according to claim 13, wherein in the compound of formula II, R_3 and R_5 are H.

21. The method according to claim 13, wherein in the compound of formula II, both R_6 and R_7 are halogens.

22. The method according to claim 13, wherein in the compound of formula II, n = 0, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

23. Use of a composition of Formula II for preparing a medicament for the treatment of cancer in mammals:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

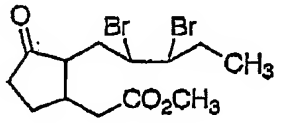
and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,
and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;
R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;
or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

24. Use according to claim 23, wherein in the compound of formula II, at least one of R_3, R_4, and R_5 is a (C_1-C_6) alkyl.

25. Use according to claim 23, wherein in the compound of formula II, R_2 is O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group.

26. Use according to claim 23, wherein in the compound of formula II, at least one of R_6 and R_7 is Br.

27. Use according to claim 23, wherein the compound of formula II is methyl jasmonate di-bromide:



28. Use according to claim 23, wherein in the compound of formula II, n=0.

29. Use according to claim 23, wherein in the compound of formula II, R_1 is a lower alkoxy.

30. Use according to claim 23, wherein in the compound of formula II, R_3 and R_5 are H.

31. Use according to claim 23, wherein in the compound of formula II, both R_6 and R_7 are halogens.

32. Use according to claim 23, wherein in the compound of formula II, $n = 0$, R_1 is a lower alkoxy, R_3 and R_5 are H, and R_6 and R_7 are halogens.

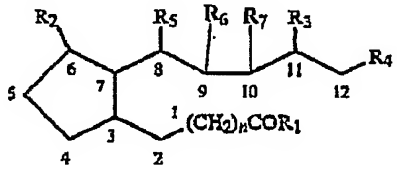
33. Use according to claim 23, wherein the medicament additionally comprises at least one active chemotherapeutic agent other than the compound of Formula II.

34. Use according to claim 23, wherein the cancer is selected from the group consisting of carcinoma, sarcoma, adenoma, hepatocellular carcinoma, hepatocellular carcinoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphagiosarcoma, synovioama, Ewing's tumor, leimyosarcoma, rhabdotheliosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hematoma, bile duct carcinoma, melanoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependynoma, pinealoma, retinoblastoma,, multiple myeloma, rectal carcinoma, cancer of the thyroid, head and neck cancer, brain cancer, cancer of the peripherial nervous system, cancer of the central nervous system, neuroblastoma, cancer of the endometrium, myeloid lymphoma, leukemia, lymphoma, lymphoproliferative diseases, acute myelocytic leukemia, chronic leukemia, Hodgkin's lymphoma, non-Hodgkins lymphoma, malignant or benign tumors of this group, and their metastasis.

35. Use according to claim 23, wherein the cancer is selected from the group consisting of prostate cancer, breast cancer, skin cancer, colon cancer, lung cancer, pancreatic cancer, lymphoma, leukemia, head and neck cancer, kidney cancer, ovarian cancer, bone cancer, liver cancer or thyroid cancer.

36. Use according to claim 23, wherein the cancer is selected from the group consisting of lymphoblastic leukemia, lung carcinoma, melanoma, and colon cancer.

37. A method for the treatment of cancer in mammals, comprising administering to a mammal a therapeutically effective amount of a pharmaceutical composition comprising as the active ingredient a compound of the Formula II:



Formula II

wherein:

n is 0,1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free

hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7;

including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

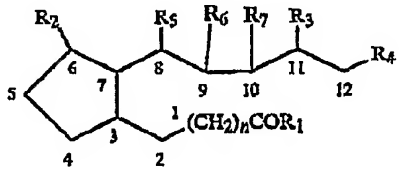
38. The method according to claim 37, wherein the cancer is selected from the group consisting of carcinoma, sarcoma, adenoma, hepatocellular carcinoma, hepatocellular carcinoma, hepatoblastoma, rhabdomyosarcoma, esophageal carcinoma, thyroid carcinoma, ganglioblastoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphagiosarcoma, synovioama, Ewing's tumor, leimyosarcoma, rhabdotheliosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hematoma, bile duct carcinoma, melanoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocyoma, medulloblastoma, craniopharyngioma, ependynoma, pinealoma, retinoblastoma,, multiple myeloma, rectal carcinoma, cancer of the thyroid, head and neck cancer, brain cancer, cancer of the peripherial nervous system, cancer of the central nervous system, neuroblastoma, cancer of the edometrium, myeloid lymphoma, leukemia, lymphoma, lymphoproliferative diseases,, acute myelocytic leukemia, chronic leukemia, Hodgkin's lymphoma, non-Hodgkins lymphoma, malignant or benign tumors of this group, and their metastasis.

39. The method according to claim 37, wherein the cancer is selected from the group consisting of prostate cancer, breast cancer, skin cancer, colon cancer, lung cancer, pancreatic cancer, lymphoma, leukemia, head and neck cancer, kidney cancer, ovarian cancer, bone cancer, liver cancer or thyroid cancer.

40. The method according to claim 37, wherein the cancer is selected from the group consisting of lymphoblastic leukemia, lung carcinoma, melanoma, and colon cancer.

41. The method according to claim 37, wherein said compound is administered at a dosage selected from 1μg/kg body weight -1000 mg/kg body weight.

42. A pharmaceutical composition for the treatment of cancer in mammals, comprising as the active ingredient a therapeutically effective amount of the compound of Formula II:



Formula II

wherein:

n is 0, 1, or 2;

R_1 is selected from OH, alkoxy, O-glucosyl and imino,

R_2 is selected from OH, alkoxy, O-glucosyl, O bound through a double bond to the carbon in position 6 thereby forming a carbonyl group, alkyl, and imino,

R_3, R_4 and R_5 are each independently selected from H, OH, alkoxy, O-glucosyl, and alkyl,

and wherein R_1 and R_2, or R_1 and R_4 may form together a lactone,

and further wherein the bonds between C_3:C_7, C_4:C_5, and C_9:C_{10} may independently from each other be double bonds or single bonds;

R_6 and R_7 are independently selected from H, Br, Fl, I, Cl, provided that least one of R_6 and R_7 is different from H;

or a derivative of said formula, wherein the derivative has at least one of the following: a lower acyl side chain at C_3 (free acid or ester or conjugate), a keto or hydroxy (free hydroxy or ester) moiety at the C_6 carbon, or an n-pentenyl or n-pentyl side chain at C_7; including salts, hydrates, solvates, optical isomers, diasteriomers, and mixtures of optical isomers thereof.

43. A method for preparation of the compound of claim 1, comprising:

ii) adding bromine to a solution of methyl jasmonate in CCL_4 under conditions appropriate for forming the compound of Formula I of claim 1;

iii) evaporating the CCL_4;

iv) obtaining the compound of claim 1.

ABSTRACT

The present invention discloses a novel jasmonate compound, and a method for its synthesis. Jasmonate compounds for pharmaceutical compositions are disclosed, especially as chemotherapeutic agents for treatment of mammalian cancers.

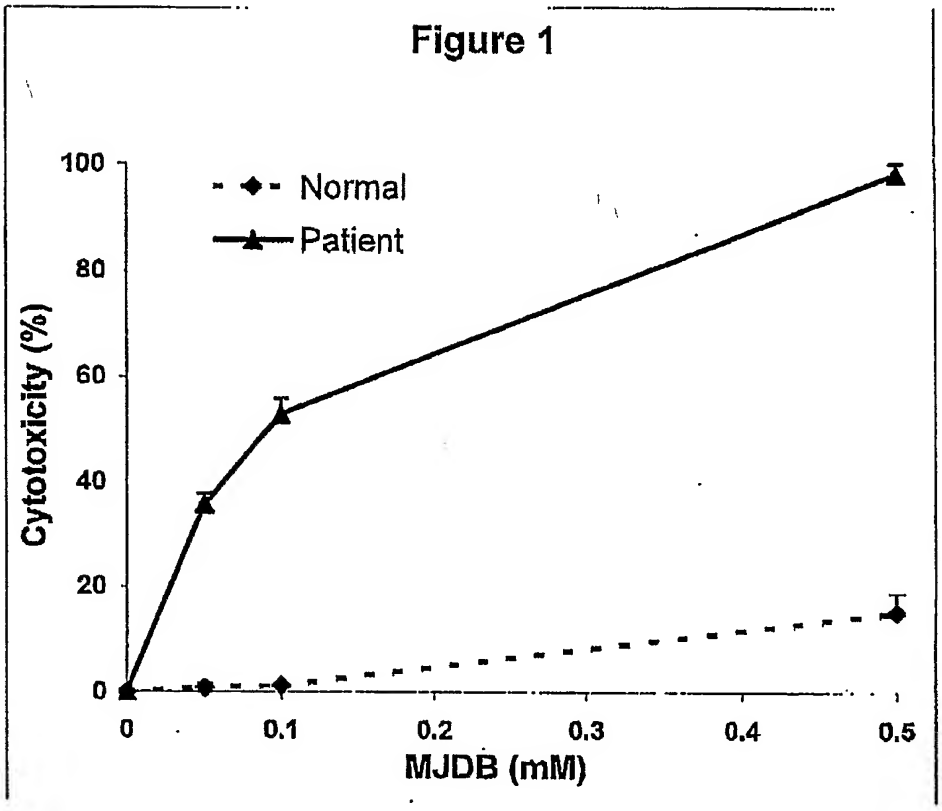


Figure 2